body in a specific direction. What is unclear is if the cell is capable of forming stable adhesions with the collagen matrix and how the force is generated. Using the modulation tracking imaging method we can follow the changes in shape of the cellular protrusion and also image separately various proteins, including actin in the cytoplasm and in the membrane. In the thin long protrusion we observe both fast diffusing actin molecules and also relatively immobile species, presumably part of the actin cytoskeleton. We are developing a method to directly measure the movement of the entire actin bundle inside the very thin cells protrusions. The method is conceptually similar to speckle imaging; however, it works in 3D.

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Super-Resolution Imaging of Chromosomal DNA in Cells

Paul D. Simonson, Eli Rothenberg, Paul R. Selvin.

Super-resolution imaging is achieved by localizing diffraction-limited spots with high accuracy. Here we combined two powerful approaches to image the chromosomal DNA inside cells. In one method, the accumulated, stochastic binding of fluorescent labels to an imaging target are localized, while in the other the fluorophore transitions between dark and bright states (compatible with binding, photobleaching, photo-activation, blinking, etc.), even when fluorophore images overlap, are localized. Combining the two techniques results in a robust microscopy that is faster than what is possible with either technique alone, requires less optimization, and corrects for cell autofluorescence. In addition, background noise due to fluorescent labels in solution can be virtually eliminated by using labels that fluoresce only when bound to the target.

Many DNA-specific dyes show dramatic fluorescence enhancement upon binding to DNA, including SYTO, LOLO, and YOYO dyes. We used nanomolar concentrations of SYTO and LOLO to image lambda DNA attached to poly-L-lysine coated glass and chromosomal DNA in fixed HEK 293 and HeLa cells. We found average single-fluorophore localization errors of 36 nm and 24 nm on glass and in cells, respectively. These imaging techniques may prove useful in future studies of chromosomal DNA in cells, including chromatin structure and defects.

This approach was further applied to imaging microtubules in vitro. We used commercial Oregon Green 488 paclitaxel to achieve a 10 nm average fluorophore localization error, and streptavidin S45A to transiently label biotinylated microtubules with Atto647N, resulting in an average of 18 nm fluorophore localization error. Future work will involve simultaneous imaging of DNA and proteins to answer important biological questions.

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Investigation of Lysosomes as Enzyme Storage Vesicles using Single Particle Tracking Fluorescence Microscopy

William H. Humphries IV, Christine K. Payne.

Intracellular, vesicle-mediated, degradation of extracellular cargo is an essential cellular function. Of particular interest is the population of vesicles responsible for degradation of extracellular cargo. Previous work using low-density lipoprotein (LDL), a classic extracellular cargo, demonstrates that the enzyme-mediated degradation of LDL occurs in a hybrid late endosomal-lysosomal vesicle[i]. In addition, the degradation of LDL occurs within 60 s of the colocalization of LDL with a lysosomal protein, LAMP1. These observations suggest that lysosomes are responsible for delivering enzymes to late endosomes, forming a hybrid organelle in which degradation occurs. Using single particle tracking fluorescence microscopy, we investigate the hypothesis that lysosomes serve as enzyme storage vesicles. We test this hypothesis using two different approaches. The first approach uses cell-permeable drugs that inhibit specific enzymes known to degrade LDL; CA074ME, a cathepsin B inhibitor, and pepstatin methyl ester, a cathepsin D inhibitor. The second approach uses siRNA to knockdown expression of LAMP. Unique to these experiments is the ability to directly monitor, in intact live cells, LDL degradation utilizing a fluorescence labeling scheme that reports on the integrity of the LDL particle. This method involves conjugation of distinct fluorophores to the protein and lipid components of LDL. Upon degradation the protein reporter decreases in fluorescence intensity while the lipid reporter increases fluorescence intensity. Our experiments make it possible to describe the complete endocytic pathway of LDL from internalization to degradation and provide a more complete picture of the intracellular degradation of extracellular cargo.

[i] W.H. Humphries IV, N.C. Fay, and C.K. Payne, *Intracellular degradation of low-density lipoprotein probed with two-color fluorescence microscopy*, Integrative Biology, in press, (2010).

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Superresolution Imaging of Intact Microbial Communities Reveals Molecular Architecture of Biofilm Development and Bacterial Organization

Veysel Berk, Nicholas Fong, Graham Dempsey, Omer Develioglu, Xiaowei Zhuang, Fitnat Yildiz, Steve Chu.

Most bacteria live as a biofilm community in their natural habitat. This surface-attached social life form is commonly found in antibiotic-resistant infections and chronic diseases. For example, bacterial biofilms are a leading cause of lung infection and death among cystic fibrosis patients. Biofilms are also crucial for bioenergy research since cellulose degrading bacteria in the gut of termites are organized as heterogenic biofilms and believed to communicate throughout these tissue-like structures. To gain structural and molecular insight on biofilm formation, we imaged intact bacterial biofilms at different developmental stages without using any fixing agents by STORM microscope. These three-dimensional superresolution images revealed ten to twenty biofilm-promoting exopolysaccharide-rich regions that are sparsely distributed on the cell surface. A few hours after surface attachment of bacteria, these small globular structures expanded to ~100 nm in size and protruded from the cell surface. During the initial stage of biofilm formation, we observed extensive interactions among neighboring cells through these globular exopolysaccharides. Moreover, we identified straight cable-like cell-to-cell and cell-to-substrate connections, up to 5 microns in length, originating from globular structures. These physical interactions may explain how bacteria form initial microcluster on the surface, first stage of commitment to biofilm formation. Microcluster formation depends on bacterial twitching motility on the surface and exopolysaccharide synthesis.These results are shifting the paradigm in the biofilm field which states that bacteria are randomly embedded in an extracellular matrix in biofilms. Our data suggests that bacteria actively build their house similar to a spider web by synthesizing sticky globular polysachharides on the cell surface, which are then extended to cable-like structures by twitching motility on the surface.

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3D Tracking of Single Fluorescent Particles with Sub-Millisecond and Nanometer Resolution

Joerg Bewersdorf, Manuel F. Juette.

Observing dynamics at the nanoscale requires sub-millisecond time resolution. Notably, in studying biological systems, three-dimensional (3D) trajectories of fluorescently labeled objects such as viruses or transport vesicles often need to be acquired with high temporal resolution.

Here, we present a novel instrument (1) which combines scanning-free multiplane detection at 3.2 kHz frame rate and single photon sensitivity with optimized beam-steering capabilities. This setup enables ultrafast 3D localization with sub-millisecond time resolution and nanometer localization precision. We demonstrate 3D tracking of single fluorescent particles at speeds of up to 150 nm/ms over several seconds and large volumes. By focused excitation of only the particle of interest - while avoiding confocal pinholes - the system realizes maximum detection efficiency at minimal laser irradiation. These features, combined with the avoidance of stage movement, provide high live-sample compatibility for future biomedical applications.

Next to the characterization of the instrument, we will show first biomedical applications.

(1) Juette, M.F. and Bewersdorf, J., Nano Letters, in press

3342-Pos Board B447

Fluorinated Membrane Potential Probes

Ping Yan, Adrian Negrean, Huibert D. Mansvelder, Leslie M. Loew.

To explore the effect of fluorination on photophysical properties of membrane potential probes, we developed synthetic methods for ANEP dyes with fluorine substitutions at the donor, bridge, and acceptor sides. Fluorination on acceptor side induces red shifts in optical spectra while on donor side it induces blue shifts. The trend can be qualitatively rationalized as electron redistribution from donor in the ground state to acceptor in the excited state, and quantitatively predicted by quantum mechanical calculations using time-dependent density functional theory (TDDFT). Compared with parent ANEP dyes, fluorinated dyes generally show improved photostabilities in addition to similar fast response kinetics and high voltage sensitivities when tested in a voltage-clamped hemispherical lipid bilayer (HLB) apparatus. The characteristics of red-shifted absorption spectra, improved photostability, and high voltage sensitivity have enabled us to achieve a single trial resolution of 50 mV in two-photon imaging of cultured hippocampal neurons using 1100-1350 nm laser excitation, paving the way for in vivo imaging of spontaneous action potentials. (Supported by NIH grants EB001963).